

## IN THE CLAIMS

1. *(Currently Amended)* A synthetic gene encoding a polypeptide segment, wherein said polypeptide segment corresponds to a reference polypeptide segment encoded by a naturally occurring polyketide synthase (PKS) gene, and

a) the polypeptide segment encoded in the synthetic gene and the polypeptide segment encoded by the naturally occurring gene are the same length and comprise at least 50 amino acids;

b) the polypeptide segment encoded in the synthetic gene and the polypeptide segment encoded by the naturally occurring gene are at least 95% identical in amino acid sequence; and

c) the polypeptide segment-encoding sequence of the synthetic gene and the polypeptide segment-encoding sequence of the naturally occurring gene are less than 90% identical in nucleotide sequence,

d) the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;

and at least one of;

e) the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

f) the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

i) a Spe I site near the sequence encoding the amino-terminus of the module;

ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;

iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;

iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;

v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;

viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

2. *(Cancelled)*

3. *(Previously Presented)* The synthetic gene of claim 1 wherein the polypeptide segment comprises a PKS domain selected from AT, ACP, KS, KR, DH, ER, and TE.
4. *(Original)* The synthetic gene of claim 3 that encodes one or more PKS modules.
5. *(Original)* The synthetic gene of claim 4 comprising at most one copy per module-encoding sequence of a restriction enzyme recognition site selected from the group consisting of Spe I, Mfe I, Afi II, Bsi WI, Sac II, Ngo MIV, Nhe I, Kpn I, Msc I, Bgl II, Bss HII, Sac II, Age I, Pst I, Kas I, Mlu I, Xba I, Sph I, Bsp E, and Ngo MIV recognition sites.
6. *(Original)* The synthetic gene of claim 1 wherein the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene.
7. *(Previously Presented)* A synthetic gene of claim 1 encoding a polypeptide segment that corresponds to a reference polypeptide segment encoded by a naturally occurring PKS gene, wherein the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:
  - a) a Spe I site near the sequence encoding the amino-terminus of the module;
  - b) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;
  - c) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;
  - d) a Msc I site near the sequence encoding the amino-terminus of an AT domain;
  - e) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;
  - f) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;
  - g) an Age I site near the sequence encoding the amino-terminus of a KR domain;
  - h) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.
8. *(Original)* A vector comprising a synthetic gene of claim 1.

9.     **(Original)** The vector of claim 8 that is an expression vector.
10.    **(Original)** A library of vectors each comprising a synthetic gene of claim 1.
11.    **(Original)** The vector of claim 8 that comprises an open reading frame encoding a first PKS module and one or more of:
- a) a PKS extension module;
  - b) a PKS loading module;
  - c) a thioesterase domain; and
  - d) an interpolypeptide linker.
12.    **(Original)** A cell comprising an expression vector of claim 9.
13.    **(Original)** The cell of claim 12 comprising a polypeptide encoded by the vector.
14.    **(Previously Presented)** The cell of claim 13 that comprises a functional polyketide synthase (PKS), wherein said PKS comprises a polypeptide encoded by said vector.
15.    **(Original)** A method of making a polyketide comprising culturing a cell of claim 14 under conditions in which a polyketide is produced, wherein the polyketide would not be produced by said cell in the absence of said vector.
- 16-68. **(Cancelled)**
69.    **(Previously presented)** The synthetic gene of Claim 1 wherein the polypeptide segment-encoding sequence of said synthetic gene is less than about 85% identical to said polypeptide segment-encoding sequence of said naturally occurring gene.
70.    **(Previously presented)** The synthetic gene of Claim 1 wherein the polypeptide segment-encoding sequence of said synthetic gene is less than about 80% identical to said polypeptide segment-encoding sequence of said naturally occurring gene.

71. ***(Previously presented)*** The synthetic gene of Claim 1 wherein the polypeptide segment-encoding sequence of said synthetic gene is less than about 70% identical to said polypeptide segment-encoding sequence of said naturally occurring gene.

72. ***(Previously presented)*** The synthetic gene of Claim 1 that is 1000-10,000 nucleotides in length.

73. ***(Previously presented)*** The synthetic gene of Claim 1 that is 3000-10,000 nucleotides in length.

74. ***(Previously presented)*** The synthetic gene of Claim 1 wherein the polypeptide segment encoded by the synthetic gene corresponds to at least 100 contiguous amino acid residues encoded by the naturally occurring gene.

75. ***(Previously presented)*** A synthetic gene encoding a polypeptide encoded by a naturally occurring polyketide synthase (PKS) gene, wherein the nucleotide sequence of the synthetic gene is less than about 90% identical to the nucleotide sequence of the naturally occurring gene.

76. ***(Currently Amended)*** The synthetic gene of Claim 1 that is produced by a method comprising:

a) providing a first set of DNA units, each in a first-type selectable vector comprising a first selectable marker and providing a second set of DNA units, each in a second-type selectable vector comprising a second selectable marker different from the first,

wherein said first-type and second-type selectable vectors can be selected based on the different selectable markers, and,

wherein ~~the each of the~~ DNA units encodes an amino acid sequence of a portion of the polypeptide segment, such that when they are joined in order the resulting synthetic gene encodes the polypeptide segment;

b) recombinantly joining a DNA unit from the first set with an adjacent DNA unit from the second set to generate a first-type selectable vector comprising a third DNA unit, and obtaining a desired clone by selecting for the first selectable marker

c) recombinantly joining the third DNA unit with an adjacent DNA unit from the second set to generate a first-type selectable vector comprising a fourth DNA unit, and obtaining a desired clone by selecting for the first selectable marker, or

recombinantly joining the third DNA unit with an adjacent DNA unit from the second series to generate a second-type selectable vector comprising a fourth DNA unit, and obtaining a desired clone by selecting for the second selectable marker.

77. *(Previously presented)* The synthetic gene of claim 1, wherein the segment-encoding sequence of the synthetic gene has the same sequence as the segment-encoding sequence of the naturally occurring gene.

78. *(Previously presented)* The gene of claim 1 wherein the polypeptide encoded by the synthetic gene has at least 95% sequence identity with the naturally occurring polypeptide.

79. *(Previously presented)* The gene of claim 1 wherein the polypeptide encoded by the synthetic gene has at least 97% sequence identity with the naturally occurring polypeptide.

80. *(Previously presented)* The gene of claim 1 wherein the polypeptide encoded by the synthetic gene has same sequence as the naturally occurring polypeptide.

81. *(Previously presented)* The synthetic gene of claim 1 wherein the reference polypeptide segment is at least 100 residues in length.

82. *(Previously presented)* The synthetic gene of claim 1 wherein the polypeptide segment-encoding sequence of said synthetic gene comprises from 500 to 50,000 basepairs.

83. *(Previously presented)* The synthetic gene of claim 82 wherein the polypeptide segment-encoding sequence of said synthetic gene is from 3000 to 10,000 basepairs.

84. *(Previously presented)* The synthetic gene of claim 82 wherein the polypeptide segment-encoding sequence of said synthetic gene is from 5000 to 20,000 basepairs.

85. *(Currently Amended)* A synthetic gene comprising from 5,000 to 50,000 basepairs encoding a polypeptide segment that corresponds to a reference polypeptide segment encoded by a naturally occurring polyketide synthase (PKS) gene, wherein the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment - encoding sequence of said naturally occurring gene, wherein

a) the polypeptide segment encoded in the synthetic gene and the polypeptide segment encoded by the naturally occurring gene are at least 97% identical in amino acid sequence; and

b) the polypeptide segment-encoding sequence of the synthetic gene and the polypeptide segment-encoding sequence of the naturally occurring gene ~~is~~ are less than 80% identical in nucleotide sequence,

c) the polypeptide segment-encoding sequence of the synthetic gene comprises at least one unique restriction site that is not present or is not unique in the polypeptide segment-encoding sequence of said naturally occurring gene, and

d) the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene; and at least one of:

de) the polypeptide segment-encoding sequence of the synthetic gene is free from at least one restriction site that is present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

f) the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

- i) a Spe I site near the sequence encoding the amino-terminus of the module;
- ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;
- iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;
- iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;
- v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

- vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;
- vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;
- viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

86. *(Currently Amended)* A synthetic gene produced by a process comprising

- a) obtaining the DNA sequence of a naturally occurring gene;
- b) obtaining a first amino acid sequence of at least 100 amino acids encoded by the naturally occurring polyketide synthase (PKS) gene;
- c) synthesizing a nucleic acid that encodes a second amino acid sequence of at least 100 amino acids, wherein
  - i) said second amino acid sequence is at least 95% identical to the first amino acid sequence; ~~and~~
  - ii) the naturally occurring sequence encoding said first amino acid sequence has less than 80% sequence identity to the synthetic sequence encoding said second amino acid sequence;

iii) the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;  
and at least one of:

iv) the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

v) the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

1) a Spe I site near the sequence encoding the amino-terminus of the module;

2) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;

3) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;

4) a Msc I site near the sequence encoding the amino-terminus of an AT domain;

5) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

6) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

7) an Age I site near the sequence encoding the amino-terminus of a KR domain;

8) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

87. ***(Previously presented)*** The synthetic gene of claim 86 wherein said second amino acid sequence is at least 97% identical to the first amino acid sequence.

88. ***(Currently Amended)*** A synthetic gene encoding a protein at least 100 amino acids in length, produced by a process comprising

a) obtaining the DNA sequence of a naturally occurring polyketide synthase (PKS) gene encoding a naturally occurring protein;

b) obtaining the first amino acid sequence of the naturally occurring protein;

c) designing an artificial DNA that has less than 80% nucleotide identity with the naturally occurring gene and which encodes a polypeptide with at least 95% sequence identity with the naturally occurring protein; and

d) synthesizing a nucleic acid comprising the sequence of the artificial DNA, wherein

i) the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;  
and at least one of:

ii) the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or



iii) the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

1) a Spe I site near the sequence encoding the amino-terminus of the module;

2) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;

3) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;

4) a Msc I site near the sequence encoding the amino-terminus of an AT domain;

5) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

6) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

7) an Age I site near the sequence encoding the amino-terminus of a KR domain;

8) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

89. ***(Previously presented)*** The synthetic gene of claim 88 wherein said second amino acid sequence is at least 97% identical to the first amino acid sequence.

90. ***(Currently Amended)*** A synthetic gene encoding a polypeptide segment, wherein said polypeptide segment corresponds to a reference polypeptide segment encoded by a naturally occurring polyketide synthase (PKS) gene, and

a) the polypeptide segment encoded in the synthetic gene and the polypeptide segment encoded by the naturally occurring gene are the same length and comprise at least 50 amino acids;

b) the polypeptide segment encoded in the synthetic gene and the polypeptide segment encoded by the naturally occurring gene are at least about 95% identical in amino acid sequence; and

c) the polypeptide segment-encoding sequence of the synthetic gene and the polypeptide segment-encoding sequence of the naturally occurring gene are less than about 90% identical in nucleotide sequence;

d) the polypeptide segment encoded in the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;

and at least one of:

e) the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

f) the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

i) a Spe I site near the sequence encoding the amino-terminus of the module;

ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;

iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;

iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;

v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;

viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

91. *(Currently Amended)* A synthetic gene that comprises a nucleotide sequence encoding a synthetic polyketide synthase (PKS) protein, said nucleotide sequence comprising from 10 kilobasepairs to 50 kilobasepairs encoding said PKS protein, wherein;

said synthetic PKS protein has an amino acid sequence that is at least 98% identical to an amino acid sequence of a PKS protein encoded by a naturally occurring gene sequence; and

wherein the nucleotide sequence of the synthetic gene has less than 80% sequence identity to said naturally occurring gene sequence;

the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;

and at least one of:

e) the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

f) the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

- i) a Spe I site near the sequence encoding the amino-terminus of the module;
- ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;
- iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;
- iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;
- v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;
- vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;
- vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;
- viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

92. *(Currently Amended)* The synthetic gene of claim 1 wherein the naturally occurring gene sequence is a sequence encoding a PKS selected from the group consisting of erythromycin PKS (SEQ ID NO: 31 or 32 ~~accession no. M63676/M63677~~); megalomicin PKS (SEQ ID NO: 33 ~~accession no. AF263245~~); oleandomycin PKS (SEQ ID NO: 34 or 35 ~~accession no. AF220951/L09654~~); pikromycin PKS (SEQ ID NO: 36 ~~accession no. AF079138~~); niddamycin PKS (SEQ ID NO: 37 ~~accession no. AF016585~~); tylosin PKS (SEQ ID NO: 38 ~~accession no. AF055922~~); pimaricin PKS (SEQ ID NO: 39 ~~accession no. AJ278573~~); pte PKS (SEQ ID NO: 40 ~~accession no. AB070949~~); avermectin PKS (SEQ ID NO: 41 ~~accession no. AB032367~~); oligomycin PKS (SEQ ID NO: 42 ~~accession no. AB070940~~); nystatin PKS (SEQ ID NO: 43 ~~accession no. AF263912~~) and amphotericin PKS (SEQ ID NO: 44 ~~accession no. AF357202~~).

93. *(Currently Amended)* A synthetic gene encoding a module of a synthetic polyketide synthase protein ([ " ]synthetic PKS module[ " ]), wherein said synthetic gene is produced by a process comprising

a) obtaining the DNA sequence of a naturally occurring PKS gene, said DNA sequence comprising from 10 kilobasepairs to 50 kilobasepairs, wherein said naturally occurring PKS gene encodes a module of a naturally occurring PKS protein ([ " ]naturally occurring PKS module[ " ]);

b) synthesizing a nucleic acid sequence that encodes the synthetic PKS module, wherein;

the amino acid sequence of the synthetic PKS module is at least 98% identical to the amino acid sequence of the naturally occurring PKS module;

~~and wherein~~ the nucleic acid sequence encoding the synthetic PKS module has less than 80% sequence identity to the nucleotide sequence of said naturally occurring PKS gene;

the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;

and at least one of;

the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of;

i) a Spe I site near the sequence encoding the amino-terminus of the module;

ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;

iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;

iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;

v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;

viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

94. **(Currently Amended)** A synthetic gene encoding a module of a synthetic polyketide synthase protein ([[]]synthetic PKS module[[]]), wherein said synthetic gene is produced by a process comprising

a) obtaining the DNA sequence of a naturally occurring PKS gene, said DNA sequence comprising from 5 kilobasepairs to 50 kilobasepairs, wherein said naturally occurring PKS gene encodes a module of a naturally occurring PKS protein ([[]]naturally occurring PKS module[[]]);

b) synthesizing a nucleic acid sequence that encodes the synthetic PKS module, wherein;

the amino acid sequence of the synthetic PKS module is at least 98% identical to the amino acid sequence of the naturally occurring PKS module;

~~and wherein~~ the nucleic acid sequence encoding synthetic PKS module has less than 80% sequence identity to the nucleotide sequence of said naturally occurring PKS gene;

the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;

and at least one of;

the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of;

- i) a Spe I site near the sequence encoding the amino-terminus of the module;
- ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;
- iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;
- iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;
- v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;
- vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;

viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.

95. ***(Currently Amended)*** A synthetic gene that comprises a nucleotide sequence encoding a synthetic polyketide synthase (PKS) protein, said nucleotide sequence comprising from 5 kilobasepairs to 50 kilobasepairs encoding said PKS protein,

wherein said synthetic PKS protein has an amino acid sequence that is at least 98% identical to an amino acid sequence of a PKS protein encoded by a naturally occurring gene sequence; and

~~wherein~~ the nucleotide sequence of the synthetic gene has less than 80% sequence identity to said naturally occurring gene sequence;

the polypeptide segment encoded by the synthetic gene retains the activity of the polypeptide segment encoded by the naturally occurring gene;

and at least one of;

the polypeptide segment-encoding sequence of the synthetic gene is free from at least one Type IIS enzyme restriction site present in the polypeptide segment-encoding sequence of said naturally occurring gene; or

the polypeptide segment-encoding sequence of the synthetic gene is different from the polypeptide segment-encoding sequence of said naturally occurring PKS gene and comprises at least two of:

i) a Spe I site near the sequence encoding the amino-terminus of the module;

ii) a Mfe I site near the sequence encoding the amino-terminus of a KS domain;

iii) a Kpn I site near the sequence encoding the carboxy-terminus of a KS domain;

iv) a Msc I site near the sequence encoding the amino-terminus of an AT domain;

v) a Pst I site near the sequence encoding the carboxy-terminus of an AT domain;

vi) a BsrB I site near the sequence encoding the amino-terminus of an ER domain;

vii) an Age I site near the sequence encoding the amino-terminus of a KR domain;

viii) an Xba I site near the sequence encoding the amino-terminus of an ACP domain.